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Basic Dye Analysis

By HPLC

Multi-Charged Dyes

The analysis of dyes is an important analytical process during dye production and in real-world dyes applications. Many dyes are produced as a mixture of several structural and constitutional isomers. After production, their purity level then needs to be established. Many dyes especially are used as food additives and therefore need to be free of possible harmful contaminants. Thus, it presents a significant problem if dyes are not properly tested. Also often a counterfeited dyes appears on the market and their testing is not trivial.

Basic dyes are important class of dye chemistry. They have at least 1 (and in many cases, more than 1) basic amine group. The strong ionic interactions make these compounds difficult to analyse utilizing traditional reverse phase HPLC mode.

To address these needs, SIELC Technologies has developed a new HPLC separation mode called BIST™ (Bridge Ion Separation Technology) that can easily separate multi-charged molecules such as these basic dye compounds.

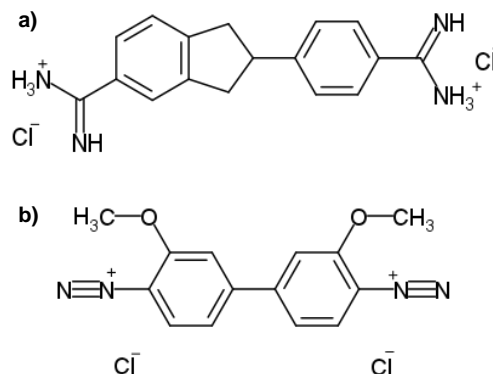
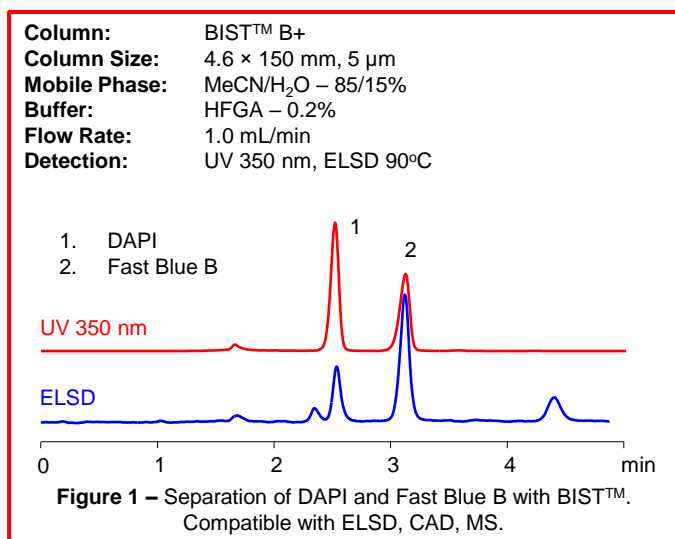
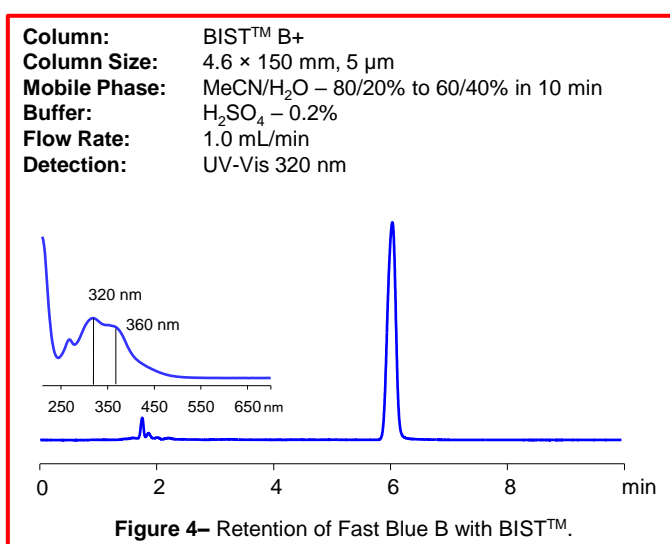
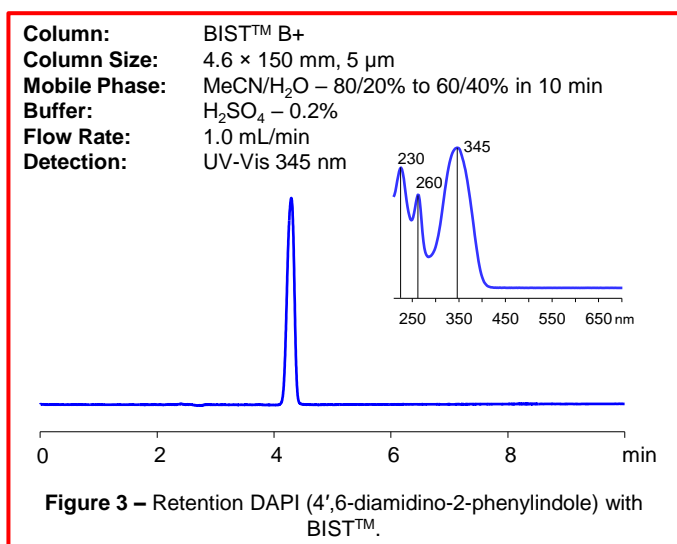


Figure 2 – Chemical structures of multi-charged dyes: a) DAPI and b) Fast Blue B salt

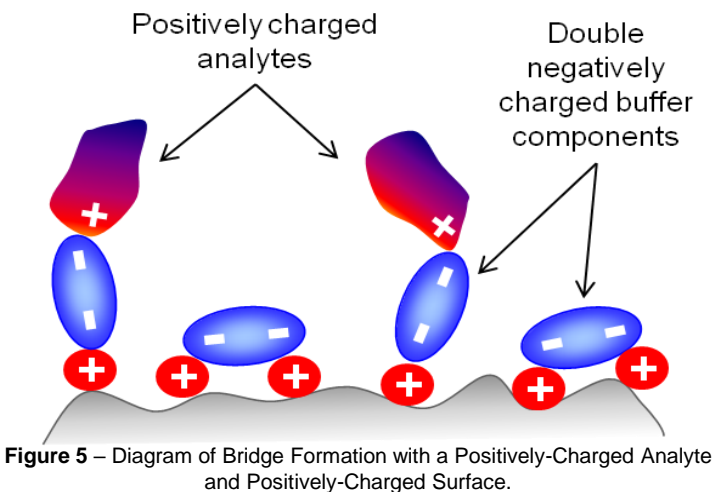
BIST™ in the study depicted in Figure 1 was used to separate and analyze the dye Fast Blue B and the fluorescent stain 4',6-diamidino-2-phenylindole (DAPI).

Typically, BIST™ methods rely on Sulfuric acid as the multi-charged ionic modifier on positively charged BIST™ columns; however, Sulfuric acid is not compatible with evaporative detection methods such as Evaporative Light Scattering Detection (ELSD), Charged Aerosol Detection (CAD), and Electrospray Ionization (ESI) for Mass Spectrometry (MS). Instead, we utilized Hexafluoroglutaric acid (HFGA), which also has a minus-2 charge in solution, but is volatile enough to be compatible with these detection methods.



BIST™ Explained

Bridge Ion Separation Technique, or BIST™, is based on the retention of charged molecules on a stationary phase of like polarity via a bridge formed between the analyte and stationary phase surface by means of a doubly-charged ionic component of the mobile phase. This doubly charged mobile phase component, which we call a bridge ion, provides the necessary electrostatic forces to retain ions on a stationary phase of the same charge polarity. This retention mechanism is expressed more profoundly in a mobile phase with reduced water content.



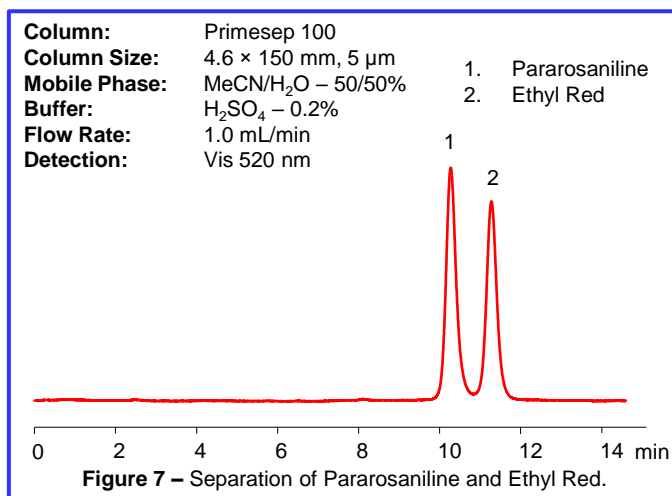
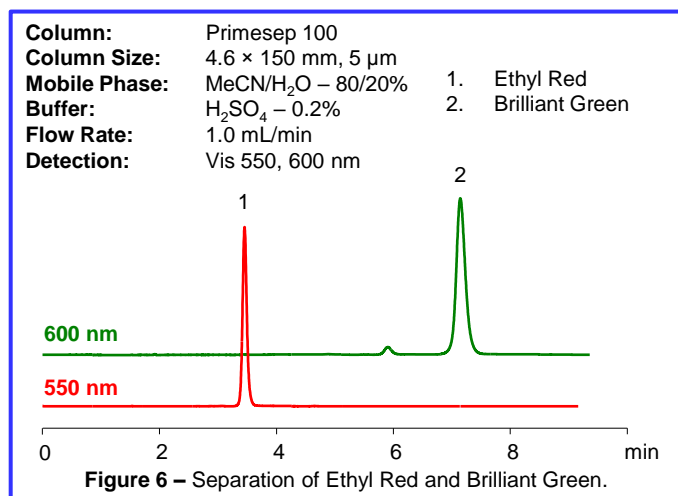
For BIST™ to work, 3 conditions need to be met:

- *A double-charged ionic modifier is present in the mobile phase*
- *The ionic modifier's double-charged ions should be opposite in charge to that of the stationary phase surface*
- *Reduced water content in the mobile phase to minimize ion solvation*

Single-Charged Dyes

SIELC has also developed HPLC methods based on mixed-mode Primesep 100 columns to analyze single-charged basic dyes. The single charge of these compounds is not sufficient to retain these compounds on BIST™ columns, so they require a different analysis method in order to be analyzed, as show in Figs. 6-9.

Mixed-mode chromatography works differently compared to BIST™ in that it provides multiple modes of interaction for charged analytes. The presence of these interactions allows chemists to use mobile phase modifications with greater flexibility than in traditional reversed-phase chromatography. Ionizable compounds can interact with the stationary phase by reverse-phase (RP), ion-exchange (IE), or ion-exclusion mechanisms. The amount of the ionic modifier in the mobile phase (i.e. buffer strength) influences the retention characteristics to the ion-exchange interaction just as the organic component affects the retention in reverse-phase separation (i.e. hydrophobic interactions). Thus, the amounts of organic and ionic MP modifiers are both important for controlling the retention of ionizable analytes on these mixed-mode columns.



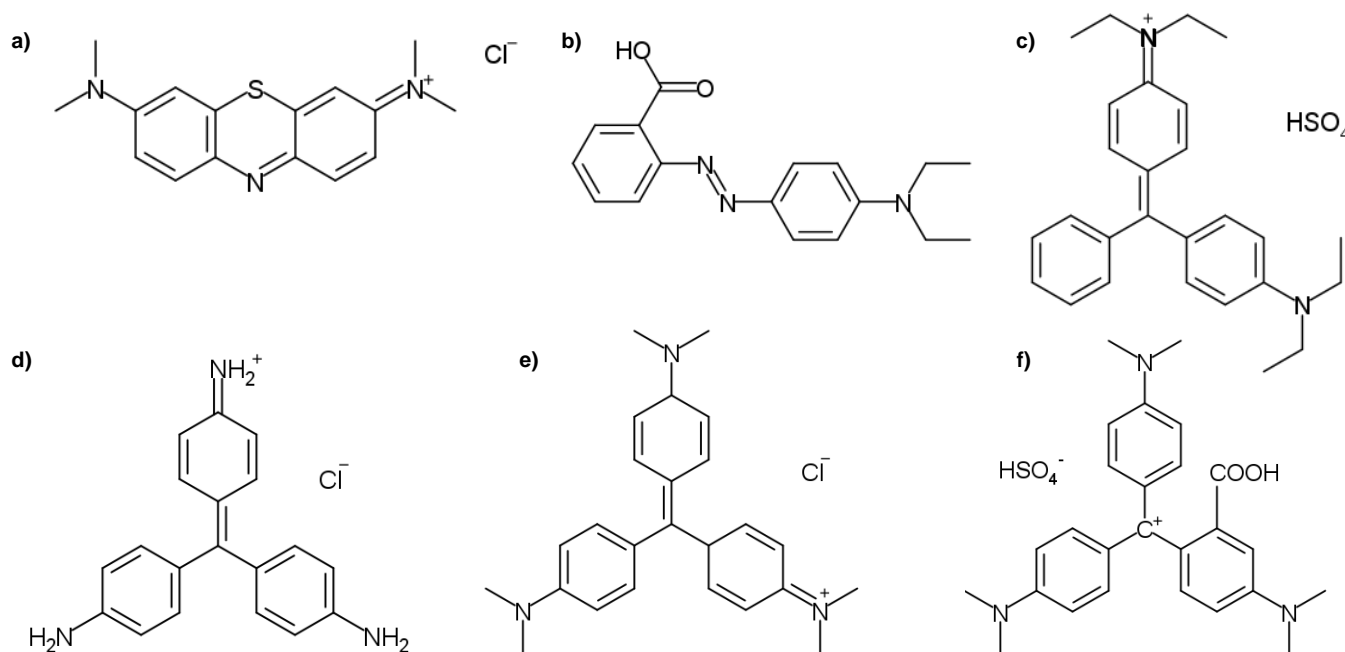
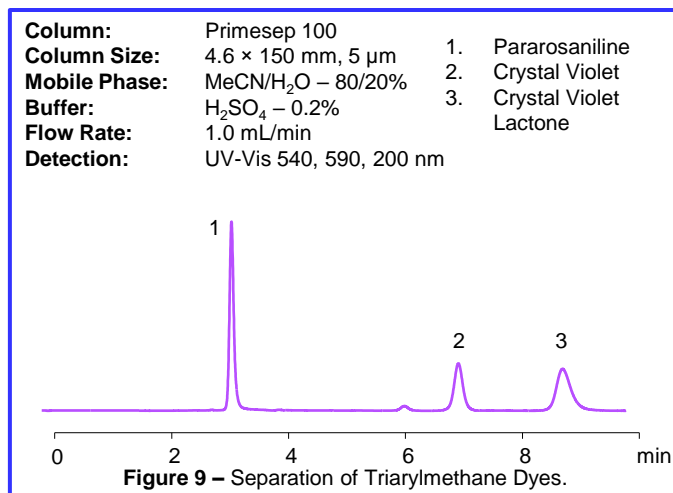
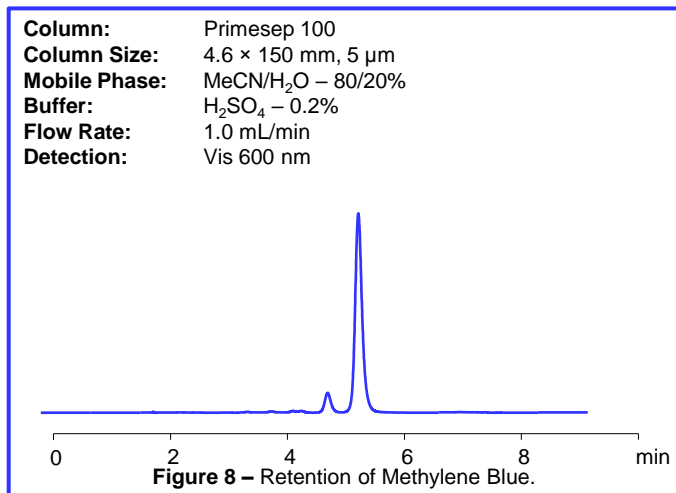



Figure 10 – Chemical structures of single-charged dyes: a) Methylene Blue b) Ethyl Red c) Brilliant Green FCF d) Pararosaniline e) Crystal Violet f) Crystal Violet Lactone



Column Part Number Generator

TBP – 32 . 100 . 0210

To order a column or ask a question send your message to sales@sielc.com or call us at : **+1 (847) 229-2629**

Type of Packing		Column ID		Column Length		Particle Size		Pore Size	
Primesep 100	100	50 mm	500	250 mm	250	2.7 μm	02	100 Å	10
BIST B+	TBP	30 mm	300	150 mm	150	3 μm	03		
		22 mm	220	100 mm	100	5 μm	05		
		10 mm	100	50 mm	50	10 μm	10		
		4.6 mm	46	25 mm	25				
		3.2 mm	32	10 mm	10				
		2.1 mm	21	Guard	G				
		1.0 mm	10						
		0.5 mm	05						

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U.S. Patents Pending. All data were obtained in SIELC Technologies labs.